Reviewer's report

Title: Regulation of Interferon Pathway in 2-Methoxyestradiol-treated Osteosarcoma Cells

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Reviewer: Dominique Heymann

Reviewer's report:

The present manuscript investigates the involvement of interferon (IFN) pathway in estrogen metabolite-induced anti-tumor effects in osteosarcoma cells. The topic of this paper is interesting and demonstrates the potential therapeutic interest of IFN pathway suspected for a long time, I have several methodological concerns about some of the experimental data presented by the authors which are summarized below.

Major compulsory revisions

Results

- Figure 1: authors showed that 2-ME treatment stimulated the luciferase activity in MG63 osteosarcoma cells. Did they obtain similar data with other human osteosarcoma cell lines which have various differentiation level. It is necessary to confirm this observation on other human cell lines.

- Figure 2: It is surprising to analyse the effect of 2-ME on IFN mRNA expression in 143B cells because this was not the cell line analyzed in Figure 1. That’s why it is necessary to show the results obtained in several human osteosarcoma cell lines. Furthermore, a dose-response of 2-ME must be shown. What was the concentration of 2ME used in Figure 2? IFNb mRNA is increased at 8h and 24h, and no modulation is observed at 16h: how do you explain this result?

- Figures 6: authors claimed that IFNa enhances 2-ME-effects in MG63 osteosarcoma cells (Fig. 6A) and in three other cell lines: the additive effect is very low excepted in HOS cell line and is mainly the consequence of 2ME. How do you explain this observation? According the p53 status of osteoc

- Figure 7: similarly to figure 6 the additive effect of 2ME and IFNb and IFNg is very low and not convincing in several cell line such as MG63

- Figures 6-7: is there any effect of combined treatment on cell cycle and apoptosis?
- Figure 8A: what is the effect of a combined treatment IFN/2-ME on peIF2a? Results obtained in the other cell lines are required.

- Figure 8B: what do the authors mean by “normal” tissue? A full description of the cohort studied is necessary (osteosarcoma subtype, age of patients, localization, etc). Are these analyses carried out after the initial biopsy or after resection en-bloc-large and then after chemotherapy? Please show immunohistochemistry of peIF2a to demonstrate its downregulation in osteosarcoma cells.

Discussion, first paragraph:

“Furthermore, IFN co-treatment enhances the 2-ME-mediated anti-tumor effects in osteosarcoma cells.” The term “anti-tumor effects” is not appropriated specifically because no pre-clinical is presented.

Several aspects of the discussion is not directly demonstrated by the results shown: for instance “Our results show that 2-ME treatment does not affect the IFN pathway in normal osteoblasts that are resistant to 2-ME-mediated anti-proliferative effects. 2-ME treatment does not induce IFN promoter activities or gene expression in normal osteoblasts. Also, 2-ME does not regulate IFN-stimulated gene pathways in normal cells. Thus, this shows that there is an association between anti-proliferative effects of 2-ME and induction of IFN pathways.” The results revealed that osteoblasts in the conditions used do not respond to 2ME. What would be the results in different differentiation medium?

Minor essential revisions

Introduction

Authors claimed in the introduction section that "Several studies have demonstrated that IFNs block osteosarcoma growth in patients, animal models and in cultured cells [10]." It is necessary to describe and comment with more details the main results available in the literature and to better justify the present work done in this context.

Materials and methods

- Describe the method used to isolate normal osteoblast
- Indicate the concentration of each antibody used for western blot

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests